

## Amino Acid Composition of $\alpha$ -Casein and $\beta$ -Casein<sup>2</sup>

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The heterogeneity of cow's milk casein has been established by solubility studies and electrophoretic analysis. Warner<sup>3</sup> reviewed earlier work on this problem and described the chemical separation of casein into two mutually distinct components,<sup>4</sup>  $\alpha$ -casein and  $\beta$ -casein, which occur in unfractionated casein in the approximate ratio of 4:1. The fractions isolated by Warner, although not electrophoretically homogeneous over the entire pH range, were purified, so that neither fraction contained any of the other. Comparison of the nitrogen and phosphorus contents of the isolated fractions with those of whole casein showed some significant differences; the values for  $\beta$ -casein, the minor component, differed from the values for whole casein more markedly than did those for  $\alpha$ -casein.

The present paper deals with the amino acid analysis of  $\alpha$ -casein and  $\beta$ -casein. Whole casein was also analyzed by the same methods for purposes of comparison. It has been possible to account for essentially all the nitrogen of each protein in terms of known amino acid residues and amide nitrogen.

### Experimental

**Proteins Used.**—The samples of whole casein,  $\alpha$ -casein and  $\beta$ -casein were prepared by Dr. Warner according to his published directions.<sup>3</sup> Electrophoretic analysis showed that each fraction was free of the other. In preliminary experiments, two preparations of each protein were analyzed for total nitrogen, phosphorus, lysine, tryptophan, tyrosine and amino nitrogen. The results indicated that there was no significant difference in composition between the two preparations. Therefore, in all subsequent experiments no distinction was made between different preparations of the same protein.

**Methods of Analysis.**—All analyses were carried out on air-dried protein samples; moisture determinations were made as suggested by Chibnall, *et al.*<sup>5</sup> True ash was de-

termined according to Warner,<sup>3</sup> total nitrogen by the Kjeldahl method as used by Miller and Houghton<sup>6</sup> and phosphorus by the method of Fiske and SubbaRow<sup>7</sup> after digestion with sulfuric and nitric acids. Amino nitrogen values were obtained by the Van Slyke method as modified by Doherty and Ogg,<sup>8</sup> and amide nitrogen was estimated in Conway micro-diffusion cells according to the procedure suggested by Warner and Cannan.<sup>9</sup> In the latter procedure, a series of Conway vessels, each containing 10 mg. protein in 1 ml. 1.5 *N* sodium hydroxide in the outer compartment and 1.5 ml. 2% boric acid in the inner chamber, was set up at 35°. At intervals which ranged from thirty-five to sixty-five hours, vessels were removed from the oven, and the distilled ammonia was titrated with 0.01 *N* hydrochloric acid. The value at each reaction time was determined in triplicate. A progressive increase in ammonia liberated with time was observed, so that a linear extrapolation of the values to zero time was made to obtain the amide nitrogen figures.

Unless otherwise noted, hydrolysis of the proteins was carried out in 6 *N* hydrochloric acid in an oil-bath at 120° for twenty hours.

Lysine was determined by means of a specific decarboxylase both on total hydrolyzates and on catholytes obtained by ionophoresis of aliquots of the same hydrolyzates. Hanke's adaptation<sup>10</sup> of Gale's method<sup>11</sup> to the Van Slyke-Neill manometric apparatus was used. Ionophoresis was employed for the primary purpose of securing catholytes suitable for photometric determinations of arginine and histidine. A three-compartment cell patterned after that of Albanese<sup>12</sup> was constructed, and the general procedure of Gordon, Martin and Synge<sup>13</sup> was followed, with hydrolyzates prepared from 0.5 g. of protein. Arginine and histidine were then determined by Macpherson's modifications<sup>14</sup> of the Sakaguchi-Weber and Pauly reactions on the catholytes obtained after repeated (4 times) ionophoresis.<sup>15</sup> The values obtained for lysine in these catholytes were consistently lower (6 to 9%) than those found in the original hydrolyzates. On the

(6) Miller and Houghton, *J. Biol. Chem.*, **159**, 373 (1945).

(7) Fiske and SubbaRow, *ibid.*, **66**, 375 (1925).

(8) Doherty and Ogg, *Ind. Eng. Chem., Anal. Ed.*, **15**, 751 (1943).

(9) Warner and Cannan, *J. Biol. Chem.*, **142**, 725 (1942).

(10) We are indebted to Prof. M. E. Hanke for details of his procedure [*Federation Proc.*, **5**, 137 (1946)], for a preparation of the enzyme and for a culture of *Bacterium cadaveris*.

(11) Gale, *Biochem. J.*, **39**, 46 (1945).

(12) Albanese, *J. Biol. Chem.*, **134**, 467 (1940).

(13) Gordon, Martin and Synge, *Biochem. J.*, **35**, 1369 (1941).

(14) Macpherson, *ibid.*, **36**, 59 (1942).

(15) After our analyses for the basic amino acids had been completed, the comprehensive paper on this scheme of analysis by Macpherson appeared.<sup>11</sup> Our experience confirms (a) the need for repeated ionophoresis of the catholyte to effect complete purification of the basic amino acid fraction, and, (b) the increased precision of the colorimetric methods when standards are used instead of calibration curves. In our hands, however, determination of lysine by difference on the basis of nitrogen analyses on the catholyte was not entirely trustworthy.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Preliminary reports of this work have been presented at meetings of the American Society of Biological Chemists, by title at the Chicago Meeting [*Federation Proc.*, **6**, 255 (1947)]; and orally at the Detroit Meeting [*ibid.*, **8**, 202 (1949)].

(3) Warner, *THIS JOURNAL*, **66**, 1725 (1944).

(4) A third component,  $\gamma$ -casein, may be present to the extent of a few per cent.; this point is discussed by McMeekin and Polis in "Advances in Protein Chemistry," Vol. V, Academic Press, Inc., New York, in press.

(5) Chibnall, Rees and Williams, *Biochem. J.*, **37**, 354 (1943).

TABLE I  
MICROBIOLOGICAL ASSAY CONDITIONS

Amino acid	Organism <sup>a</sup>	Basal medium	Standard amino acid <sup>b</sup>	Range <sup>c</sup> , standard curve used	Hours of incubation at 37°
Glycine	<i>L. mesenteroides</i> P-60	Shankman <sup>29</sup>		25-100 <sup>d</sup>	48
Valine	<i>L. arabinosus</i> 17-5	Stokes <sup>28</sup>	DL	15-80	48-96
Leucine	<i>S. faecalis</i> R	Stokes <sup>28,e</sup>	L	15-80	40-48
Isoleucine	<i>S. faecalis</i> R	Stokes <sup>28,e</sup>	DL	10-70	40-48
Proline	<i>L. mesenteroides</i> P-60	Henderson <sup>30</sup>	L	25-50	120
Phenylalanine	<i>L. arabinosus</i> 17-5	Henderson <sup>30</sup>	DL	10-60	48
Aspartic acid	<i>L. mesenteroides</i> P-60	Henderson <sup>30</sup>	L	20-70	96
Glutamic acid	<i>L. arabinosus</i> 17-5	Stokes <sup>28,f</sup>	L	10-60	48

<sup>a</sup> Obtained from the American Type Culture Collection. <sup>b</sup> Special samples of L-leucine and DL-isoleucine were obtained from Merck and Company through the courtesy of Dr. E. E. Howe; stated purity of the L-leucine was at least 99% and of the DL-isoleucine at least 96%, as determined by solubility tests. Other standard amino acids were of the "Analytically or Chemically Pure Grade" of Amino Acid Manufacturers, University of California, Los Angeles. <sup>c</sup> In micrograms of L-amino acid per tube containing a final volume of 10 ml. <sup>d</sup> Range shown does not include glycine added to basal medium to overcome induction period. <sup>e</sup> Modified by the addition of 250 mg. sodium citrate per tube containing a final volume of 10 ml. in order to enhance growth and acid production. <sup>f</sup> Modified by the substitution of L-asparagine for aspartic acid.<sup>32</sup>

assumption that mechanical losses involved in repeated ionophoresis accounted for this discrepancy, the lysine determinations on original hydrolyzates were accepted as more nearly correct, and a correction based on lysine recovery was applied to all arginine and histidine values.

Tyrosine and tryptophan were determined on alkaline stannite hydrolyzates according to the Brand and Kassell adaptation<sup>16</sup> of the Millon-Lugg procedure. The corrections recommended by Brand and Kassell were applied to the results. Also, tryptophan analyses were made on the unhydrolyzed proteins by the glyoxylic acid reaction as applied by Shaw and McFarlane.<sup>17</sup> The tryptophan values obtained by the two methods were in good agreement.

Cystine determinations were made, by Kassell and Brand's modification<sup>18</sup> of the phosphotungstic acid reaction, on hydrochloric acid and hydrochloric acid-formic acid hydrolyzates, and by Vassel's adaptation<sup>19</sup> of the Fleming reaction, on hydrochloric acid-formic acid hydrolyzates, with concordant results. The occurrence of cysteine in casein is unlikely.<sup>20</sup>

Methionine was determined both as volatile iodide and as homocysteine after hydrolysis with hydriodic acid according to the Kassell and Brand modification<sup>21</sup> of Baernstein's method. The correction factors of Kassell and Brand were applied.

Serine was determined by oxidation with periodate and distillation of formaldehyde by the technique of Boyd and Logan<sup>22</sup> and estimation of formaldehyde photometrically with chromotropic acid according to MacFadyen.<sup>23</sup> Threonine was estimated by periodate oxidation and diffusion of acetaldehyde in Conway vessels by the method of Winnick.<sup>24</sup> Correction factors for decomposition of serine and threonine during acid hydrolysis have been worked out by Rees<sup>25</sup> for mixtures of amino acids. We have assumed that the factors (100/89.5 for serine and 100/94.7 for threonine) were applicable to hydrolyzates of casein and its fractions, and the serine and threonine values have been so corrected. The corrected figures for serine may still be low, in view of the greater lability to acid hydrolysis of serine combined as phosphoserine in phosphoproteins, as compared with that of free serine.

Thus, Nicolet, *et al.*, give 7.38% as the probable serine content of casein, compared with 5.5% found on direct acid hydrolysis.<sup>26</sup> A correction factor based on these figures would be 100/74.5.

Glycine, valine, leucine, proline, phenylalanine, aspartic acid and glutamic acid were determined by microbiological assay. Our technique in general was patterned after that of Stokes and co-workers,<sup>27,28</sup> but differed with respect to the following minor variations. Cells for inoculum were suspended in a small volume of physiological saline and added to the basal medium previously sterilized by filtration through a Seitz filter. Five-ml. portions of the mixture were then pipetted into tubes containing 5-ml. aliquots of either standard amino acid or unknown, previously sterilized by autoclaving. Standards were run in quadruplicate at 10 levels of amino acid concentration and unknowns in quadruplicate at 5 levels. Lactic acid production was determined by titration (glass electrode). Conditions used in the assays are summarized in Table I; further deviations from published procedures are noted there.

Attempts to work out a microbiological assay method for alanine from suggestions in the literature<sup>34-36</sup> were unsuccessful. Likewise, attempts to determine alanine by the Alexander and Seligman method in its original form<sup>37</sup> or as applied to protein hydrolyzates by Michel and Michel<sup>38</sup> were unsatisfactory. Our values for alanine were obtained by the method of Aqvist,<sup>39</sup> which involves deamination to lactic acid, conversion of the lat-

(10) Brand and Kassell, *J. Biol. Chem.*, **131**, 489 (1939).

(17) Shaw and McFarlane, *Can. J. Research*, **16B**, 361 (1938).

(18) Kassell and Brand, *J. Biol. Chem.*, **125**, 115 (1938).

(19) Vassel, *ibid.*, **140**, 323 (1941).

(20) Kassell and Brand, *ibid.*, **125**, 435 (1938).

(21) Kassell and Brand, *ibid.*, **125**, 145 (1938).

(22) Boyd and Logan, *ibid.*, **146**, 279 (1942).

(23) MacFadyen, *J. Biol. Chem.*, **158**, 107 (1945).

(24) Winnick, *ibid.*, **142**, 461 (1942).

(25) Rees, *Biochem. J.*, **40**, 632 (1946).

(26) Nicolet, Shinn and Saidel, Buffalo Meeting, Am. Chem. Soc., Sept., 1942, Abstracts, p. 22B.

(27) Stokes and Gunness, *J. Biol. Chem.*, **157**, 651 (1945).

(28) Stokes, Gunness, Dwyer and Caswell, *ibid.*, **160**, 35 (1945).

(29) Shankman, Camien and Dunn, *J. Biol. Chem.*, **168**, 51 (1947).

(30) Henderson and Snell, *ibid.*, **172**, 15 (1948).

(31) Teply and Elvehjem, *ibid.*, **157**, 303 (1945).

(32) Rabinowitz and Snell, *ibid.*, **169**, 631 (1947).

(33) Baumgarten, Mather and Stone, *Cereal Chem.*, **22**, 514 (1945).

(34) Brand, Saidel, Goldwater, Kassell and Ryan, *THIS JOURNAL*, **67**, 1524 (1945).

(35) Buehler, Schantz and Lamanna, *J. Biol. Chem.*, **169**, 295 (1947).

(36) Knight, *J. Exptl. Med.*, **86**, 125 (1947); *J. Biol. Chem.*, **171**, 297 (1947); we wish to thank Dr. Knight for a culture of the strain of *Streptococcus faecalis* R used in his alanine determinations.

(37) Alexander and Seligman, *J. Biol. Chem.*, **159**, 9 (1945).

(38) Michel and Michel, *Bull. soc. chim. biol.*, **29**, 886 (1947).

(39) Aqvist, *Acta Physiol. Scand.*, **13**, 297 (1947).

ter to acetaldehyde and determination of the aldehyde with *p*-hydroxybiphenyl. The figures should be regarded only as approximations.

**Comments on Methods.**—Vickery<sup>40</sup> has stressed the need for maintaining certain specialized standards of accuracy in amino acid analysis of proteins. In conforming to these standards insofar as possible, we have made liberal use of the "standard proteins,"  $\beta$ -lactoglobulin and bovine serum albumin, and of mixtures of amino acids, for control analyses. Each method of analysis was tested on a "standard protein" before it was adopted for use. In the microbiological assays, an analysis of lactoglobulin was made simultaneously with every run on unknown protein. With few exceptions, our results on lactoglobulin and serum albumin agreed closely with the analyses listed by Brand.<sup>41</sup>

Table II shows the divergent values for lactoglobulin, together with other figures from the recent literature. Alanine analyses of lactoglobulin by the Aqvist method, although they average 7.8%, a not unreasonable value, are not included in the table because the results were erratic.

TABLE II  
AMINO ACID ANALYSES OF LACTOGLOBULIN, G./100 G. OF PROTEIN

	Brand <sup>41</sup>	Values from present work	Other values
Glycine	1.4	1.8	1.56 (Keston, <i>et al.</i> <sup>42</sup> )
Isoleucine	8.4	7.1	5.86 (Stein and Moore <sup>43</sup> )
Proline	4.1	4.8	4.84 (Keston, <i>et al.</i> <sup>42</sup> )
Serine	5.0	4.3	4.07 (Rees <sup>25</sup> )
Threonine	5.85	5.3	5.11 (Rees <sup>25</sup> )

It will be obvious, especially to protein analysts, that to obtain results in agreement with reliable values in the literature within a few per cent., although reassuring, is no proof of accuracy. This is particularly true of the serine, threonine and other determinations which involve correction for destruction during hydrolysis, of the amide nitrogen determination, and of some of the microbiological procedures. Therefore analyses of casein,  $\alpha$ -casein and  $\beta$ -casein were carried out under identical conditions and simultaneously whenever possible, so that observed differences in composition could be accepted as real, at least in a comparative sense.

### Discussion

Averaged analytical results are listed in Table III. All have been corrected for moisture. Since true ash amounted to less than 0.4% in each protein, it was disregarded in the calculations. Corrections previously mentioned have been applied to the figures for methionine, tryptophan, arginine, histidine, serine, threonine and tyrosine.

(40) Vickery, *Ann. N. Y. Acad. Sci.*, **47**, 63 (1946).

(41) Brand, *ibid.*, **47**, 187 (1946).

(42) Keston, Udenfriend and Cannan, *THIS JOURNAL*, **71**, 249 (1949).

(43) Stein and Moore, *J. Biol. Chem.*, **176**, 337 (1948).

$\alpha$ -Casein and  $\beta$ -casein differ in their content of most of the amino acids. Most striking, perhaps, are the observed differences in proline, tryptophan and tyrosine content, and the presence of less than 0.1% cystine in  $\beta$ -casein. Histidine, glutamic acid, threonine and, obviously, amide nitrogen are considered to be present in equal concentration within experimental error. The differences in glycine, isoleucine and serine content, although not large, are considered as probably significant, especially since any differences in composition relative to casein would be accentuated in  $\beta$ -casein, the minor component. Definite conclusions regarding alanine cannot be drawn from the available data; the figures for this amino acid are included only to show its approximate concentration.

There have been previous reports that casein fractions differ in phosphorus, tyrosine and tryptophan content but, as Warner<sup>3</sup> has pointed out, it is unlikely that the fractions analyzed were homogeneous. However, the alcohol-soluble casein containing little phosphorus, which was isolated by Osborne and Wakeman<sup>44</sup> and by Linderström-Lang<sup>45</sup> may well be a distinct component of casein, with its own characteristic amino acid composition.<sup>4</sup>

In more recent studies, Mellander<sup>46</sup> has reported that  $\alpha$ -casein, prepared by Warner's method, contained 6.8% serine, whereas his original casein contained 3.5%. And in an abstract by Hagberg and Swanson<sup>47</sup> it is stated that " $\alpha$ -casein contained a higher percentage of total phosphorus, aspartic acid, glutamic acid and tyrosine" (than  $\beta$ -casein, being implied). Our data, except for glutamic acid, are in accord with the latter statement. The comparison of Mellander regarding serine, however, seems questionable because of the low value of 3.5% for casein (*cf.* Rees<sup>25</sup>) and because of the following considerations.

If it is assumed that casein is composed of only  $\alpha$ -casein and  $\beta$ -casein, and if the analyses for any common constituent differ significantly in the three proteins, then the relative amounts of  $\alpha$ -casein and  $\beta$ -casein in casein may be calculated. Thus, using the figures for tyrosine in Table III, 6.3, 8.1 and 3.2%, one obtains a ratio of 63:37 for the proportion of  $\alpha$ -casein to  $\beta$ -casein in casein. This calculation has been made for 14 of the constituents listed in Table III; the resulting ratios range from 58:42 to 78:22 and average 69:31. The ratio deduced by Warner from electrophoretic patterns was 80:20. Mellander's values do not fit any reasonable ratio of this kind, but if the value of 3.5% serine in casein is indeed low, the figure of 6.8% in  $\alpha$ -casein is possible.

The completeness of the analyses as shown by the summations of nitrogen in Table III might

(44) Osborne and Wakeman, *J. Biol. Chem.*, **33**, 243 (1918).

(45) Linderström-Lang, *Compt. rend. trav. lab. Carlsberg*, **17**, No. 9, 116 pp. (1929).

(46) Mellander, *Uppsala Läkarefören. Förh.*, **52**, 107 (1947).

(47) Hagberg and Swanson, *J. Dairy Sci.*, **31**, 718 (1948).

TABLE III  
COMPOSITION OF WHOLE CASEIN,  $\alpha$ -CASEIN AND  $\beta$ -CASEIN

Constituent	Whole casein	$\alpha$ -Casein, g./100 g. protein	$\beta$ -Casein	Whole casein, g. amino acid	$\alpha$ - Casein N/100 g. protein N	$\beta$ - Casein N/100 g. protein N
Total N	15.63	15.53	15.33			
Total P	0.86 <sup>a</sup>	0.99 <sup>a</sup>	0.61 <sup>a</sup>			
Amino N	0.93 (0.92-0.94) [4,1] <sup>b</sup>	0.99 (0.97-1.00) [4,1]	0.72 (0.71-0.73) [4,1]			
Glycine	2.7 (2.59-2.85) [3,5]	2.8 (2.66-2.85) [3,5]	2.4 (2.28-2.41) [2,5]	3.2	3.4	2.9
Alanine	3.0 <sup>c</sup> (2.0-4.0) [4,3]	3.7 <sup>c</sup> (2.2-4.1) [4,3]	1.7 <sup>c</sup> (0.5-3.1) [4,3]	3.0 <sup>c</sup>	3.7 <sup>c</sup>	1.7 <sup>c</sup>
Valine	7.2 (7.02-7.48) [3,5]	6.3 (6.18-6.37) [3,5]	10.2 (9.89-10.40) [2,5]	5.5	4.9	8.0
Leucine	9.2 (8.86-9.46) [2,5]	7.9 (7.79-8.07) [2,5]	11.6 (11.57-11.71) [2,5]	6.3	5.4	8.1
Isoleucine	6.1 (6.06-6.23) [3,5]	6.4 (6.27-6.56) [3,5]	5.5 (5.23-5.68) [3,5]	4.2	4.4	3.8
Proline	11.3 (10.89-11.53) [4,5]	8.2 (8.04-8.31) [4,5]	16.0 (15.60-16.40) [2,5]	8.8	6.4	12.7
Phenylala- nine	5.0 (4.88-5.07) [2,5]	4.6 (4.47-4.67) [3,5]	5.8 (5.62-6.05) [3,5]	2.7	2.5	3.2
Cystine	0.34 <sup>d</sup>	0.43 <sup>d</sup>	0.0-0.1 <sup>d</sup>	0.3	0.3	...
Methionine	2.8 <sup>e</sup>	2.5 <sup>e</sup>	3.4 <sup>e</sup>	1.7	1.5	2.1
Tryptophan	1.2 <sup>f</sup>	1.6 <sup>f</sup>	0.65 <sup>f</sup>	1.1	1.4	0.6
Arginine	4.1 (3.98-4.19) [2,3]	4.3 (4.24-4.37) [2,3]	3.4 (3.24-3.48) [2,3]	8.4	8.9	7.1
Histidine	3.1 (2.94-3.18) [2,3]	2.9 (2.80-3.03) [2,3]	3.1 (3.11-3.14) [2,3]	5.4	5.1	5.5
Lysine	8.2 (8.11-8.18) [3,2]	8.9 (8.85-8.95) [3,2]	6.5 (6.47-6.57) [3,2]	10.1	11.0	8.1
Aspartic acid	7.1 (6.78-7.46) [3,5]	8.4 (8.10-8.65) [3,5]	4.9 (4.70-5.09) [3,5]	4.8	5.7	3.4
Glutamic acid	22.4 (21.10-24.40) [4,5]	22.5 (21.10-25.20) [5,5]	23.2 (22.40-23.90) [2,5]	13.6	13.8	14.4
Amide N	1.6 (1.60-1.61) [2,1]	1.6 (1.59-1.64) [3,1]	1.6 (1.60-1.66) [3,1]	10.2	10.3	10.4
Serine	6.3 (6.15-6.41) [4,3]	6.3 (6.20-6.38) [5,3]	6.8 (6.76-6.89) [2,3]	5.4	5.4	5.9
Threonine	4.9 (4.68-5.05) [6,3]	4.9 (4.69-5.11) [7,3]	5.1 (4.98-5.17) [3,3]	3.7	3.7	3.9
Tyrosine	6.3 (6.00-6.84) [17,2]	8.1 (7.82-8.28) [13,2]	3.2 (3.09-3.30) [7,2]	3.1	4.0	1.6
Total	115.8 <sup>g</sup>	115.7 <sup>g</sup>	117.4 <sup>g</sup>	101.5	101.8	103.4

<sup>a</sup> These values are identical with those reported by Warner.<sup>3</sup> <sup>b</sup> Figures in brackets show the number of determinations, followed by the number of duplicative analyses in each determination; figures in parentheses are averages of the duplicative analyses and show the range of the individual determinations from which the final average value was calculated; in the microbioassays, analyses at 5 levels of unknown concentration are considered as duplicative analyses. <sup>c</sup> These values are provisional. <sup>d</sup> The figures listed are averaged results of two methods; found for casein by Brand-Kassell method, 0.35(0.30-0.39)[6,2]; and by Vassel method, 0.33(0.30-0.34)[6,2]; found for  $\alpha$ -casein, by Brand-Kassell method 0.42(0.40-0.49)[7,2] and by Vassel method 0.43(0.41-0.45)[5,2]; less than 0.1% cystine was found in  $\beta$ -casein by either method. <sup>e</sup> Averaged results of 2 methods; found for casein by volatile iodide, 2.85(2.63-3.05)[5,2], and as homocysteine, 2.73(2.65-2.80)[5,1]; found for  $\alpha$ -casein, 2.55(2.49-2.62)[4,2] and 2.53(2.47-2.66)[4,1]; found for  $\beta$ -casein, 3.42(3.38-3.46)[2,2] and 3.45(3.44-3.46)[2,1]. <sup>f</sup> Averaged results of 2 methods; found for casein by Brand-Kassell method, 1.23(1.06-1.37)[17,2] and by Shaw-McParlane, 1.26(1.18-1.32)[12,2]; found for  $\alpha$ -casein, 1.46(1.32-1.53)[13,2] and 1.65(1.56-1.75)[11,2]; found for  $\beta$ -casein, 0.74(0.72-0.77)[7,2] and 0.55(0.46-0.65)[5,2]. <sup>g</sup> Total includes amino acids, amide N calculated as ammonia (1.9%), and phosphorus calculated as phosphoric acid (2.7, 3.1 and 1.9%, respectively, for casein,  $\alpha$ -casein and  $\beta$ -casein).

conceivably be the result of numerous compensating errors. The presence of other amino acids or other groups must be considered possible, although it is reasonably certain that they could not be present in large concentration. Additional evidence for the essential completeness of the analyses may be found in the summations by McMeekin, *et al.*,<sup>48</sup> of amino acid residue weights, 98.13, 98.12 and 99.15, for whole casein,  $\alpha$ -casein and  $\beta$ -casein, respectively.

The values herein reported for the amino acid composition of whole casein are compared with figures from the literature (Table IV) in order to demonstrate that casein is fairly well characterized in spite of its heterogeneity. The selection of data from the great number of published values is admittedly arbitrary, as is also the omission of hydroxyproline and citrulline.

(48) McMeekin, Groves and Hipp, *THIS JOURNAL*, **71**, 3298 (1949).

To show correlations between physical properties and amino acid composition, the data in Table III have been recalculated in terms of side chain groups (Table V). The treatment follows that of Brand, *et al.*,<sup>34</sup> with respect to grouping of side chain residues and calculating free  $\alpha$ -amino, free  $\alpha$ -carboxyl, free glutamic acid carboxyl and glutamine groups. It has been assumed that all the phosphorus is present as phosphoserine, the excess of serine being listed as free serine groups. The phosphoserine side chains are considered dibasic anionic groups. Incidentally, if they are considered monobasic anionic groups, and if the free  $\alpha$ -amino and free  $\alpha$ -carboxyl groups are not included in the summations, the figures for total groups become 845, 837 and 873 moles of amino acids per 10<sup>5</sup> g. of whole casein,  $\alpha$ -casein and  $\beta$ -casein, respectively; the reciprocals of these totals, 118.3, 119.5 and 114.5, are

TABLE IV  
AMINO ACID COMPOSITION OF WHOLE CASEIN, G./100 G.  
OF PROTEIN

	Values from present work	Values from literature
Glycine	2.7	1.9 <sup>49</sup>
Alanine	3.0	3.5 <sup>49</sup>
Valine	7.2	7.2 <sup>30</sup>
Leucine	9.2	10.3 <sup>30</sup>
Isoleucine	6.1	7.6 <sup>30</sup>
Proline	11.3	11.6 <sup>30</sup>
Phenylalanine	5.0	5.5 <sup>30</sup>
Cystine	0.34	0.34 <sup>30</sup>
Methionine	2.8	3.1 <sup>20</sup>
Tryptophan	1.2	1.2 <sup>50</sup>
Arginine	4.1	4.0 <sup>51</sup>
Histidine	3.1	3.2 <sup>51</sup>
Lysine	8.2	8.3 <sup>51</sup>
Aspartic acid	7.1	7.2 <sup>52</sup>
Glutamic acid	22.4	22.0 <sup>53</sup>
Amide N	1.6	1.4 <sup>25</sup>
Serine	6.3	5.9 <sup>25</sup>
Threonine	4.9	4.6 <sup>25</sup>
Tyrosine	6.3	6.2 <sup>30</sup>
Total	112.8	115.0

then the respective approximate average residue weights.

An important difference in the properties of  $\alpha$ -casein and  $\beta$ -casein, which has been utilized in this Laboratory in their separation, is the greater solubility of  $\beta$ -casein in ethanol-water mixtures. The larger proportion of non-polar groups in  $\beta$ -casein (Table V) may well account for this.

Differences in the electrophoretic mobilities of  $\alpha$ -casein and  $\beta$ -casein observed by Warner<sup>3</sup> may be explained also on the basis of amino acid composition. In solutions, both acid and alkaline to the isoelectric points of the proteins,  $\alpha$ -casein had the higher mobility. The higher proportions of cationic and anionic groups in  $\alpha$ -casein fit in with this observation. The greater concentration of phosphoric ester residues in  $\alpha$ -casein is also noteworthy because of the particular contribution of these groups to the higher mobility of  $\alpha$ -casein in alkaline solution.

A correlation between amino acid composition and specific volume for each of these proteins is demonstrated by McMeekin, *et al.*<sup>48</sup>

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(49) Tristram, *Biochem. J.*, **40**, 721 (1946).

(50) Sullivan and Hess, *J. Biol. Chem.*, **155**, 441 (1944).

(51) Macpherson, *Biochem. J.*, **40**, 470 (1946).

(52) Hac and Snell, *J. Biol. Chem.*, **159**, 291 (1945).

(53) Bailey, Chibnall, Rees and Williams, *Biochem. J.*, **37**, 360 (1943).

TABLE V  
SIDE CHAIN GROUPS IN WHOLE CASEIN,  $\alpha$ -CASEIN AND  $\beta$ -CASEIN

Group	Equiv./10 <sup>5</sup> g. protein		
	Whole casein	$\alpha$ -Casein	$\beta$ -Casein
<b>Cationic groups</b>			
Arginine	24	25	20
Histidine	20	19	20
Lysine	56	61	44
Free $\alpha$ -amino	10	10	7
Total cationic groups	110	115	91
<b>Anionic groups</b>			
Aspartic	53	63	37
Free glutamic	38	39	44
Free $\alpha$ -carboxyl	10	10	7
Phosphoserine	56	64	40
Total anionic groups	157	176	128
Total ionic groups	267	291	219
<b>Non-ionic polar groups</b>			
1/2 Cystine	3	4	0
Methionine	19	17	23
Tryptophan	6	8	3
Tyrosine	35	45	18
Free serine	32	28	45
Threonine	41	41	43
Glutamine	114	114	114
Total non-ionic polar groups	250	257	246
Total polar groups	517	548	465
<b>Non-polar groups</b>			
Glycine	36	37	32
Alanine	34	42	19
Valine	61	54	87
Leucine	70	60	88
Isoleucine	47	49	42
Phenylalanine	30	28	35
Proline	98	71	139
Total non-polar groups	376	341	442
Total groups	893	889	907

### Summary

A comparative analysis of the amino acid composition of whole casein and its two major components,  $\alpha$ -casein and  $\beta$ -casein, has been made. Within the experimental error of the analytical methods, all the nitrogen of each protein has been accounted for in terms of known amino acids and amide nitrogen.

$\alpha$ -Casein and  $\beta$ -casein differ considerably in their content of many amino acids, and these differences are reflected in such physical properties as solubility and electrophoretic mobility.

Although heterogeneous, whole casein is a fairly well characterized protein with respect to amino acid composition.